CONTROLS ON MICROBIAL PROCESSING OF DISSOLVED ORGANIC MATTER IN BOREAL FOREST STREAMS

By

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Abstract

In the boreal forest, permafrost thaw is resulting in changes in vegetation and deepening of watershed flowpaths. Caribou-Poker Creeks Research Watershed contains sub-catchments underlain with varying permafrost extents (4-53% cover), providing the opportunity to study how permafrost extent affects water chemistry and nutrient cycling. I measured nitrogen (N), phosphorous (P), and carbon (C) processing ectoenzyme activity in the water column and sediment of headwater streams, and related ectoenzyme activity to nutrient and dissolved organic carbon (DOC) concentration. Additionally, I used nutrient diffusing substrata (NDS) to grow biofilms with enhanced inorganic N and P and labile C alone and in combination and measured ectoenzyme activity and respiration of biofilms in response to resource amendments. High P-processing enzyme activity across streams of the CPCRW indicated microbial P limitation. Respiration and organic matter processing enzymes of biofilms grown on NDS increased with labile C or labile C in combination with nutrient additions, implying that labile C limited or co-limited rates of DOM processing. Our results suggest that as climate warming and subsequent permafrost thaw alters terrestrial inputs of dissolved organic matter (DOM) and inorganic nutrients into streams, changes in inorganic P and labile C availability will control microbial processing of DOM.

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Preface

This thesis is formatted as a manuscript. Chapter 1 is an introduction summarizing ecological stoichiometry and biogeochemistry of boreal forest steams. Chapter 2, Controls on Microbial Processing of Dissolved Organic Matter in Boreal Forest Streams, is a manuscript describing my primary research and written for publication. Chapter 3 is a general conclusion, discussing the implications of changing stream water chemistry of boreal forest streams.

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Chapter 1: General Introduction

1.1 Permafrost Controls Stream Chemistry

Permafrost covers 25% of the Earth's land area, and in high latitude landscapes controls vegetation growth, soil nutrient cycling, and hydrologic flowpaths between terrestrial and stream ecosystems (Anisimov and Nelson 1996, Hobbie et al. 1999, Wickland et al. 2007). The boreal forest of North America spans the southern boundary of permafrost extent, with discontinuous permafrost often underlying valley bottoms and north facing slopes. Discontinuous permafrost landscapes are susceptible to climate warming-induced permafrost thaw (Brown et al. 2016, Biskaborn et al. 2019), with the potential to transform terrestrial-stream interactions and alter inputs of dissolved organic matter (DOM) and inorganic nutrients into streams (McClelland et al. 2007, Frey and McClelland 2009, Kendrick et al. 2018).

In discontinuous permafrost landscapes, the presence and extent of permafrost underlying stream catchments determines subsurface flowpath depth (Kawahigashi et al. 2004, Lynch et al. 2019). Flowpath depth influences soil properties and controls abiotic and biotic processing of solutes as they are transported from land to streams (Ma et al. 2019). Permafrost thaw, and consequently deepened flowpaths and mobilization of solutes stored in the inorganic soil layer, is predicted to increase inorganic nutrient concentrations in streams (Hobbie et al. 1999, Frey et al. 2007a, 2007b). Additionally, interaction with the mineral soil layer will likely increase DOM sorption, favoring the retention of hydrophobic compounds in terrestrial soils (Kaiser et al, 1996), and affecting DOM composition in streams (Kawahigashi et al. 2004). Deeper flowpaths may also increase groundwater discharge into streams, with predicted DOM inputs of relatively low aromatic and high hydrophilic content (O'Donnell et al. 2016). Shallow flowpaths through soils underlain with permafrost prevent percolation of DOM into deeper soil

layers. Poor drainage can result in long residence times with increased opportunity for microbial processing to alter DOM composition in route to streams (Wickland et al. 2007). Shallow flowpaths that constrict water to surface soil layers also leads to flashy stream discharge and DOM flushes during storms (Petrone et al. 2006).

In the boreal forest of interior Alaska, poorly drained and nutrient-poor permafrost-underlain soil have black spruce (*Picea mariana*) stands, whereas permafrost-free hillslopes are covered in deciduous stands dominated by quaking aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*) (Viereck et al. 1983). Catchment vegetation can influence DOM composition (Harms et al. 2016). Plant-derived DOM is altered as it is exposed to biotic processing and abiotic retention in soils (Wickland et al. 2007), and permafrost-driven differences in vegetation stand and soil properties will likely yield DOM with different compositions in streams.

1.2 Stream Chemistry Controls DOM Processing

Microbial processing of DOM is determined by environmental C:N:P availability relative to microbial C:N:P biomass composition and metabolic requirements (Sterner and Elser 2002, Allen and Gillooly 2009). When a resource is in short supply relative to microbial demand, microbes allocate nutrients and C towards acquiring the limiting resource, decreasing C and nutrient assimilation efficiencies and inhibiting growth (Sinsabaugh and Moorhead 1994). Biofilms, heterogeneous microbial communities attached to rocks and sediment, are responsible for the majority of DOM processing that occurs in streams (Battin et al. 2016). Within biofilms, populations specialized for different functions vary in C and nutrient requirements, resulting in multiple resources simultaneously limiting stream metabolism (Battin et al. 2016).

DOM composition and nutrient content is important in meeting microbial resource requirements. The size and structure of molecules that compose DOM determine the energetic

cost of processing. Initial C and N investment towards ectoenzyme production and energy requirements to breakdown recalcitrant molecules can limit microbial processing of DOM (Allison and Vitousek 2005). Additionally, DOM composition can inhibit ectoenzyme activity, restricting use of organic nutrients (Mann et al. 2014). Microbial community composition is also important for DOM processing. Microbes specialize in processing specific substrates and how well suited microbial communities are to process available DOM determines if DOM is processed (Judd et al. 2007, D'Andrilli et al. 2019).

In streams, heterotrophic metabolism is tightly linked to inorganic nutrient concentration. Inorganic nutrients enter streams through terrestrial inputs, sourced from weathering of minerals and soil organic matter. Subsequently, nutrient cycling in streams further alters inorganic nutrient concentration. The elemental composition of microbial biomass relative to the composition of DOM determines if nutrients from DOM are mineralized into the environment or immobilized (Manzoni et al. 2008, Cotner et al. 2010). Additionally, photosynthetic algae compete with heterotrophs for inorganic nutrients (Joint et al. 2002), and nitrifiers can rapidly transform ammonium to nitrate, a less energetically favorable form of inorganic N (Findlay and Sinsabaugh 2003). Inorganic nutrients can be quickly assimilated by heterotrophic microbes (Kirchman 1994), and are often critical in meeting microbial nutrient demand (Cross et al. 2005, Cotner et al. 2010, Manzoni et al. 2017).

1.3 Ectoenzymes Mediate DOM Decomposition

Microbes secrete a variety of ectoenzymes into the environment to catalyze the processing of organic molecules and target C, N, and P acquisition. Beta glucosidase (BG) catalyzes cellulose processing for C acquisition. β-1,4- N-acetylglucosaminidase (NAG) catalyzes processing of chitin and peptidoglycan and leucine amino peptidase (LAP) catalyzes

amino acid processing. NAG and LAP collectively target the primary sources of organic N. Phosphatase (PHOS) catalyzes the release of phosphates from nucleic acids and phospholipids, which are the main sources of labile organic P (Sinsabaugh et al. 2012). Because these enzymes facilitate terminal reactions that create assimilable products and process some of the most abundant molecules in organic matter, they are useful as indicators of C, N and P enzymatic acquisition (Sinsabaugh 1994, Sinsabaugh et al. 2008, Moorhead et al. 2016).

Microbes regulate enzyme activity from environmental signals that communicate environmental substrate availability in relation to growth requirements (Sinsabaugh and Shah 2012). Numerous studies have demonstrated that inorganic P availability suppresses PHOS activity (Olander and Vitousek 2000, Hill et al. 2010b, Williams et al. 2012, Mann et al. 2014). The relationship between N availability and N processing enzyme activity is less straightforward, confounded by a multitude of enzymes that process N, a variety of N-acquiring strategies, and enzymes that process N and C simultaneously (Sinsabaugh and Shah 2012). However, studies have observed suppression of N acquiring enzymes when simple forms of N are available (Allison and Vitousek 2005, Olander and Vitousek 2000, Sinsabaugh et al. 2002). Beta-glucosidase, which catalyzes cellulose degradation, is most strongly regulated by cellulose substrate availability (Sinsabaugh et al. 2008)

Ratios of C:N:P processing enzymes, indicating relative effort towards C, N, and P acquisition, integrate the stoichiometric balance between resource availability and biomass composition with C and nutrient use (Sinsabaugh et al. 2009). Resource allocation models have used enzyme activity measurements to infer relative nutrient demand by applying the assumptions that total enzyme activity is proportional to microbial production, N and P processing enzyme activities are inversely related to N and P supply, and microbes allocate to

enzymes in order to maximize community productivity (Sinsabaugh and Moorhead 1994). Globally, the ratio of C:N:P processing enzymes are similar across soils and freshwater sediments, presumably reflecting an equilibrium between environmental C:N:P availability, microbial biomass, and the efficiency of nutrient and C assimilation (Sinsabaugh et al. 2008, 2009). Deviations from this equilibrium have since been used to detect N and P limitations on microbial community metabolism (Hill et al. 2010a, Williams et al. 2012, Burpee et al. 2016, Pastor et al. 2019).

In addition to the enzymes discussed above, phenol oxidase (POX), necessary for lignin degradation, is also commonly studied to gain insight into DOM decomposition processes (Sinsabaugh 2010, Hill et al. 2012, Sieczko and Peduzzi 2014, Pastor et al. 2019). POX is often uncorrelated to other C-processing enzymes, suggesting different mechanisms influence activity regulation (Sinsabaugh 2010). POX activity can be quite high relative to BG in high latitude waters (Mann et al. 2014), indicating the significance of lignin processing for C and nutrient acquisition. Phenolic compounds within DOM are complex and energy-intensive to break-down (Eriksson et al. 1990, Higuchi 1990, Sinsabaugh and Shah 2011) and POX processing can be a limiting step in DOM decomposition (Freeman et al. 2001, Mann et al. 2014). Because POX targets more complex compounds compared to BG, POX activity relative to BG activity has been proposed as an index of DOM recalcitrance (Sinsabaugh and Shah 2011) and can provide information into the relative importance of different C sources to community metabolism (Sieczko and Peduzzi 2014).

1.4 Consequences of Permafrost Thaw on DOM Processing

Climate change is leading to permafrost thaw, altering terrestrial vegetation and soil properties and subsequently changing stream chemistry. Long-term trends indicate that DOC and

nitrate concentrations are increasing in arctic Alaska streams (Kendrick et al. 2018). Shifts in stream C, N and P availability may alter current stoichiometric balances between the elemental makeup up of the environmental bioavailable resource pool and microbial resource requirements. Alleviation of resource limitations will increase the efficiency of C and nutrient use and increase DOM processing. Relating ectoenzyme activity to stream water chemistry informs what resources control allocation toward organic nutrient acquisition and relative C, N, and P demand (Burpee et al. 2016, Pastor et al. 2019). Changes in allocation towards ectoenzyme activities in response to predicted changes in inorganic nutrient and labile C availability can thus inform projections about how boreal forest permafrost thaw will affect microbial organic matter use and resource limitations, and consequently DOM cycling in streams (Fig. 1.1).

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1.6 Figures

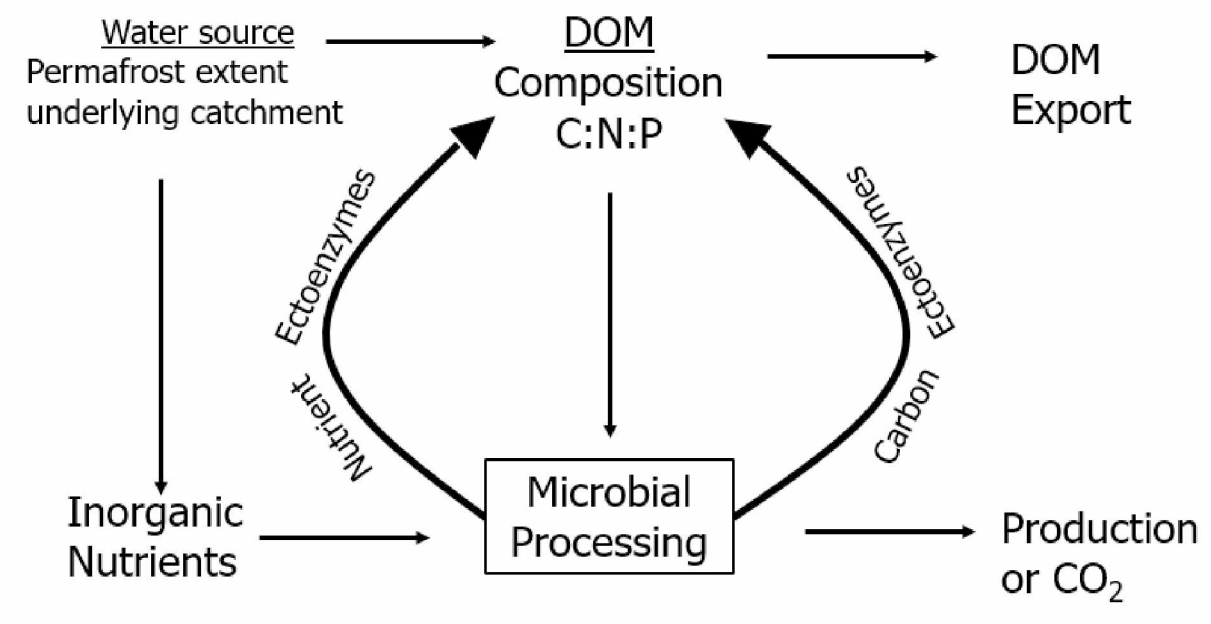

Figure 1.1 Conceptual diagram illustrating relationship between resource availability, ectoenzyme activities, and microbial processing of DOM. Permafrost extent underlying the catchments of streams affects stream inorganic nutrients and DOM molecular composition and nutrient content, and thus resources available for microbial processing. Ectoenzymes are used to process DOM. Relating C and nutrient processing enzyme activities to resource availability infers how stream chemistry affects microbial resource demand and organic matter use, important in determining the fate of DOM.

Chapter 2: Controls on Microbial Processing of Dissolved Organic Matter in Boreal Forest Streams[1]

2.1 Abstract

In the boreal forest, permafrost thaw is resulting in changes in vegetation and deepening of watershed flowpaths. Caribou-Poker Creeks Research Watershed (CPCRW) contains sub-catchments underlain with varying permafrost extents (4-53% cover), providing the opportunity to study how permafrost extent affects water chemistry and nutrient cycling. We measured nitrogen (N), phosphorous (P), and carbon (C) processing ectoenzyme activity in the water column and sediment of headwater streams, and related ectoenzyme activity to nutrient and dissolved organic carbon (DOC) concentrations. Additionally, we used nutrient diffusing substrata to grow biofilms with enhanced inorganic N and P and labile C alone and in combination, and measured ectoenzyme activity and respiration of biofilms in response to resource amendments. High P-processing enzyme activity across streams of the CPCRW suggested microbial P limitations. Respiration and organic matter processing enzyme activities of biofilms grown on NDS increased with labile C or labile C in combination with nutrient additions, implying that labile C limited or co-limited rates of DOM processing. Our results suggest that as climate warming and subsequent permafrost thaw alters terrestrial inputs of dissolved organic matter (DOM) and inorganic nutrients into streams, increases in inorganic P and labile C availability will increase microbial processing of DOM

[1]Schmidt, M. F., and J. B. Jones. 2020. Controls on Microbial Processing of Dissolved Organic Matter in Boreal Forest Streams. To be submitted to Freshwater Sciences

2.2 Introduction

Headwater streams serve as important links between terrestrial dissolved organic matter (DOM) sources and DOM transport and transformation downstream (Cole et al. 2007, Raymond et al. 2013, Hotchkiss et al. 2015). C and nutrient cycling are tightly linked, with DOM processing contingent on environmental resource availability meeting microbial nutrient and C demand. Water chemistry, including DOM molecular composition, organic nutrient content, and inorganic nutrient concentration, determines the stoichiometric balance of bioavailable C, nitrogen (N), and phosphorus (P), and constrains rates of DOM processing (Woodward et al. 2012, Mutschlecner et al. 2017, D'Andrilli et al. 2019). The boreal forest stores one third of the global terrestrial C stock (Pan et al. 2011). Boreal forest terrestrial-stream interactions and how stream chemistry controls microbial processing of DOM is important in predicting the fate of this C.

In the boreal forest, discontinuous permafrost underlays stream catchments with permafrost extent influencing stream chemistry. Flowpaths are constricted to the active layer (layer of soil that thaws seasonally), and flowpaths depth affects how solutes are mobilized and transported to streams. Permafrost thaw, and consequently deepened flowpaths and flow through the inorganic soil layer, is predicted to increase inorganic nutrient concentrations in streams (Hobbie et al. 1999, Frey et al. 2007b, 2007a). Allochthonous DOM composition is affected by terrestrial abiotic and biotic processing. Active layer depth also affects sorption processes and residence times as DOM is transported to streams, altering DOM composition (Kawahigashi et al. 2004, Balcarczyk et al. 2009, Wickland et al. 2012). Permafrost, especially at its southern boundary where permafrost is discontinuous, is thawing rapidly, bringing to question the implications of altered stream chemistry on stream C and nutrient cycling.

Terrestrial to stream export of DOM and inorganic nutrients is influenced by permafrost extent underlaying stream catchments, as well as temporal variation of snowmelt, seasonal deepening of the active layer, and rain storms (Buffam et al. 2001, Kawahigashi et al. 2004, Petrone et al. 2006, Balcarczyk et al. 2009, Mutschlecner et al. 2018). A range of controls have been observed on microbial processing of DOM in high latitude streams including inorganic and organic P (Peterson et al. 1985, Corning et al. 1989, Mutschlecner et al. 2017), inorganic N (Wickland et al. 2012, Pastor et al. 2019) and labile C (Robbins et al. 2017).

Microbes produce ectoenzymes to externally process organic molecules for C, N, and P assimilation with the activity affected by environmental resource availability (Sinsabaugh and Shah 2012). Enzyme activity informs what molecules microbes are processing and what resources microbes are investing energy towards acquiring. Microbes downregulate nutrient processing enzyme activity in response to nutrient availability (Chróst and Overbeck 1987, Foreman et al. 1998, Olander and Vitousek 2000, Sinsabaugh et al. 2002) and allocation towards C, N, and P processing enzyme activity implies relative nutrient demand (Sinsabaugh and Moorhead 1994). Ectoenzyme activity measurements integrate environmental C:N:P availability with metabolic C, N, and P assimilation (Sinsabaugh et al. 2009) and the ratio of C:N:P acquiring enzymes has been applied to determine nutrient limitations on microbial activity (Sinsabaugh et al. 2009, Hill et al. 2010b, 2014, Moorhead et al. 2013). Relating ectoenzyme activities to stream chemistry informs what resources control the balance of available C and nutrients relative to microbial nutrient requirements, and thus dictate the rate of DOM processing and the fate of C and nutrients within DOM (Burpee et al. 2016, Pastor et al. 2019).

We evaluated how stream chemistry, specifically the availability of inorganic nutrient, organic nutrient, and labile carbon, affect microbial processing of DOM. Our research was

conducted in interior Alaska, where permafrost is discontinuous and currently thawing (Osterkamp 2007, Brown et al. 2016, Biskaborn et al. 2019). We measured ectoenzyme activities in streams draining catchments underlain with contrasting permafrost extents for two summers to infer how permafrost driven and seasonal differences in water chemistry influence what resources affect microbial processing of DOM. Additionally, we measured ectoenzyme activity and microbial respiration in response to inorganic nutrient and labile C manipulations. Manipulative experiments allowed us to evaluate how specific resources affect organic matter use and relative C, N, and P demand.

2.3 Methods

2.3.1 Site Description

Caribou-Poker Creeks Research Watershed (CPCRW) is a nearly pristine research watershed located 50 km NE of Fairbanks, AK. Climate of the CPCRW is continental with warm summers (mean = 16.4 °C in July), cold winters (mean = - 29 °C in January), and low annual precipitation (411 mm/year). South-facing upland slopes are dominated by hardwood forests of Alaskan paper birch (*Betula neoalaskana*) and quaking aspen (*Populus tremuloides*), whereas north-facing slopes generally have black spruce (*Picea mariana*) and feather mosses (*Pleurozium schreberi* and *Hylocomium schreberi*). Alders are found throughout the uplands (*Alnus viridis*) and valley bottoms (*A. incana*). Valley bottoms are dominated by mosses (e.g., *Sphagnum* spp., *Hylocomium*) and dwarf shrubs (e.g., *Betula nana, Vaccinium uliginosum*).

The CPCRW is underlain with discontinuous permafrost, with permafrost found on north facing slopes and poorly drained valley bottoms. Our sampling sites included four headwater streams (C1, C2, C3, C4) draining catchments underlain with permafrost extents ranging from 4% - 53% (Fig. 2.1). In the CPCRW, streams draining catchments underlain with

more extensive permafrost tend to have higher DOC concertation, while streams draining medium permafrost catchments haver higher nitrate concentration (Petrone et al. 2006).

2.3.2 Study Design

Ectoenzymes are released into the environment by microbes and mediate DOM decomposition. Microbes induce enzyme activity based on environmental signals conveying substrate availability and resource demand, and activity thus provides information into what organic molecules microbes are potentially using and which resources are most in demand. We measured four enzymes that target C, N, and P acquisition. Beta glucosidase (BG) breaks down cellulose to acquire C. Beta-1,4- N-acetylglucosaminidase (NAG) processes chitin and leucine amino peptidase (LAP) processes amino acids, collectively used to acquire the most common forms of organic N. Phosphatase (PHOS) cleaves ester-phosphate bonds for P acquisition. Because these enzymes process some of the most abundant forms of organic matter into assimilable products, they are commonly used as indicators to infer relative effort towards C, N, and P acquisition and thus C, N, and P demand (Sinsabaugh et al. 2009, Hill et al. 2010b). Additionally, we measured phenol oxidase (POX) activity, an enzyme that specializes in the energetically costly process of lignin degradation. POX activity provides information into microbial utilization of recalcitrant molecules (Sinsabaugh and Shah 2011).

We measured water chemistry, and both water column (2018 and 2019 field season) and sediment (2019 field season) ectoenzyme activities biweekly from the four study streams. Additionally, we manipulated inorganic nutrients and labile carbon concentrations in three headwater streams using nutrient diffusing substrata (NDS), and measured ectoenzyme activity and respiration of nutrient and carbon amended biofilms. *In situ* resource manipulations allowed

us to infer resource limitations on microbial respiration and to measure microbial ectoenzyme activity responses to specific resources.

2.3.3 Stream Chemistry Analyses

In order to relate ectoenzyme activities to stream water chemistry, water and sediment samples were collected from each stream, C1, C2, C3, and C4, bi-weekly for water chemistry and ectoenzyme activity analyses. Inorganic nutrients measured were soluble reactive P (SRP), nitrate, nitrite, and ammonium. Organic nutrients were dissolved organic N (DON) and dissolved organic P (DOP). For DOC properties, we measured concentration, $SUVA_{254}$ (Weishaar et al. 2003) and spectral slope ratio (Helms et al. 2008). We filtered samples to 1.0 μm within 12 hours of collection. Enzyme activity, $SUVA_{254}$, and slope ratio were measured within 24 hours of collection. All other water chemistry analyses were either done within 48 hours of sample collection or samples were frozen for later analysis. In addition to bi-weekly grab samples for chemistry and enzyme analysis, we sampled headwater streams daily throughout the 2018 and 2019 summer using an ISCO autosampler. Daily measurements were used to evaluate stream chemistry throughout the summer and compare chemistry between streams.

DOC and TDN concentrations were measured as non-purgeable organic carbon using a Shimadzu TOC 5000 analyzer plumbed to an Antek 7050 nitric oxide chemiluminescent detector. We quantified anions (NO_3^-, NO_2^-) and cations (NH_4^+) with ion chromatography (Thermo Scientific Dionex DX-320). We calculated DON as TDN minus inorganic nitrogen concentrations (nitrate, nitrite, and ammonium). SRP was quantified using the molybdate blue method with a spectrophotometer (Shimadzu, UV Mini; 5 cm cell path, LOQ 0.7 $\mu gP\ L^{-1}$), and total dissolved P (TDP) was measured as SRP following persulfate digestion (Murphy and Riley 1962). DOP was calculated as TDP minus SRP. We measured $SUVA_{254}$ and slope ratio using a

spectrophotometer (Shumadzu UVmini-1240 1 cm cell path), to infer information about molecular composition of DOM. $SUVA_{254}$, UV absorbance at 254 nm normalized to DOC concentration, is positively correlated to DOM aromaticity (Weishaar et al. 2003, Spencer et al. 2012). Spectral slope ratio (SR) (spectral slope at 275-295 nm divided by spectral slope at 350-400nm) is negatively correlated to the molecular weight of colorimetric DOM (Helms et al. 2008).

Stream discharge was calculated throughout the summer field season by continuously measuring stream stage height with pressure transducers (Solinst Levelogger, model 3001). Rating curves were created for each stream using weekly salt slug discharge measurements against measured stage height. Continuous discharge was estimated from fitting rating carves to stage height data. Continuous discharge was not collected at C1; however, slug discharge measurements were done on biweekly enzyme sampling days.

2.3.4 Enzyme assays

Water and sediment samples were assayed fluorometrically for PHOS, BG, NAG, and LAP activity. Samples were incubated with 4-methylumbelliferone (MUB) or 7-amino-4-methylcoumarin (MUC) linked substrates in 96-well black microplates. Potential enzyme activity is quantified as the amount of fluorescence emitted from enzymes reacting with substrate and releasing MUB or MUC molecules, with activity monitored overt time in order to determine activity rate at substrate saturation. We used 4-MUB - phosphate as a substrate to measure PHOS activity, 4-MUB-b-D-glucoside to measure BG activity, 4-MUB-N-acetyl-b-glucosaminide to measure NAG activity, and L-Leucine 7-amido-4-MUC to measure LAP activity. Fluorescence

was measured using a Bio-Tek plate reader at 360 nm/460 nm emission and excitation wavelengths.

For sediment samples, 0.1 g of wet sediment, 200 µL of 5 mM sodium bicarbonate buffer, and 50 µL of 200 µM enzyme substrate were combined in 96-well black microplates. Sample controls included 0.1 g sample and 200 µL of 5 mM bicarbonate buffer, substrate controls included 200 µL of 5 mM bicarbonate buffer and 50 µL of substrate, standards included 0.1 g sample, 200 µL 5 mM bicarbonate buffer and 50 µL of MUB or MUC standard, and standard controls included 200 µL of buffer and 50 µL of MUB or MUC standard. Samples, standards, and controls were all measured in replicates of nine. Quenching, the decrease in fluorescence from interactions of enzyme substrates with non-reactant chemicals in the assay, was accounted for by comparing quenched standards, sample and MUB, with non-quenched standards, buffer and MUB. The emission coefficient, moles of MUB or MUC per fluorescent unit, was calculated from the standard control wells and corrected for quenching. Plates were read every hour for four hours. Activity was calculated as moles MUC or MUB g dry weight^{-1} hr^{-1} according to Colorado State University enzymes in the environment protocol (http://enzymes.nrel.colostate.edu).

For surface water, 200 µL of water sample and 50 µL of 200 µM substrate were mixed in microplate wells. Sample controls included of 200 µL sample and 50 µL of 5 mM bicarbonate buffer, substrate controls included 200 µL buffer and 50 µL of substrate, standards included 200 µL sample and 50 µL of 10 mM MUB or MUC standard, and standard controls included 200 µL of buffer and 50 µL 10 mM MUB or MUC standard. Samples, standards, and controls were all measured in triplicate. The emission coefficient was calculated from fluorescence measurements of standard wells. We measured fluorescence periodically for twenty-four hours. Activity was

calculated as moles MUC or MUB ml^{-1} hr^{-1}, according to Colorado State University enzymes in the environment protocol (http://enzymes.nrel.colostate.edu).

Surface water POX activity was assayed using an absorbance-based method, reacting samples with L-3,4-dihydroxyphenylalanine (DOPA). A 5 mM DOPA solution was made using nanopure water. Clear, flat-bottom microplates were used. Samples wells included 200 µL of water sample combined with 50 µL of 5 mM DOPA, control wells contained 200 µL samples and 50 µL nanopure water, and blank wells included 200 µL nanopure water combined with 50 µL of 5 mM DOPA. Samples, controls, and blanks were assayed in triplicate. Absorbance was read after 24 hours at 460 nm using a Bio-Tek plate reader. Activity was calculated as µmol hr^{-1} ml^{-1} using an extinction coefficient of 7.9 for DOPA according to Colorado State University enzymes in the environment protocol (http://enzymes.nrel.colostate.edu).

2.3.5 Nutrient Diffusing Substrata

We used nutrient diffusing substrata (NDS) to measure enzyme activity in response to specific solutes. NDS were constructed using 30 ml plastic cups with a ~2.5 cm^2 hole drilled in lid. Cups were filled with an agar nutrient substrate, and a fritted glass filter disk was placed under lid. NDS substrate was enriched with ammonium, phosphate, and acetate, alone and in combination. Treatments included a control with unamended agar (U), ammonium (N), acetate (C), phosphate (P), ammonium and acetate (N+C), phosphate and acetate (P+C), and ammonium, phosphate, and acetate (N+P+C), with five replicates per treatment. Acetate ($C_2H_3NaO_2$), ammonium (NH_4Cl), and phosphate (KH_2PO_4) were added at concentrations of 0.5 M using a 2% agar solution for single nutrient or C treatments and a 3% agar solution for treatments

containing multiple nutrient or C additions. We deployed NDS in the C1, C3, and C4 streams. NDS were secured in streams for 18 days for colonization by biofilms.

Colonized NDS disks were transported to the lab, where respiration and enzyme activities were measured within 24 hours of collection. We performed respiration incubations in 30-mL plastic falcon tubes filled with stream water collected from the mainstem of Caribou Creek. Biofilms were incubated for three hours at room temperature and in the dark, and initial and final dissolved oxygen (DO) concentrations were measured using a handheld O_2 meter (YSI PRODO). Falcon tubes containing stream water alone were included to account for respiration occurring in the incubation water. We measured NDS biofilms for BG, PHOS, NAG, and LAP activity. Biofilms were scrubbed from glass disks with a metal brush and diluted into 10 ml stream water from the main stem of Caribou Creek. Enzyme assays were performed using the same protocol as surface water enzyme assays. Quenching was accounted for by comparing fluorescence of a quenched standard, MUB or MUC standard mixed with diluted biofilm, with a non-quenched standard, MUB or MUC standard in buffer, and used to correct the emission coefficient. Samples and controls were measured in triplicate. Activity was calculated as moles MUC or MUB cm^{-2} NDS disk hr^{-1}.

2.3.6 Data Analysis

We used R (version 3.4.3) to test for between stream differences in water chemistry and enzyme activities and analyze the relationship between stream water chemistry and enzyme activity. We used ANOVAs to test for significant between stream differences in mean water chemistry measurements for 2018 and 2019, and Tukey's tests to determine which streams significantly differed. Significant differences in enzyme activities and activity ratios were also

determined using ANOVAs, with Tukey's tests determining which streams significantly differed. We evaluated the relationships between stream chemistry and ectoenzyme activity and activity ratios using Spearman rank correlation, a non-parametric test to avoid error from non-normally distributed data.

For the NDS experiment, we used ANOVAs to distinguish if respiration, enzyme activity, or enzyme activity ratios significantly differed among nutrient and C treatments and Tukey's tests to distinguish which treatments significantly differed from the unamended control. We used linear regression models to evaluate the relationship between NAG + LAP vs PHOS activity for NDS biofilms grown in each stream and determine how nutrient treatments affected investment towards P relative to N acquisition. Differences in the slope of the NAG+LAP vs PHOS regression models between treatment groups (i.e., P vs non-P treated biofilms) were determined by testing for a significant interactions of treatment group. We also used linear regression models to analyze the relationship of NDS biofilm enzyme activities relative to respiration for each stream. We tested for difference in slope between streams with a homogeneity of regression slope test. If no significant difference was detected we evaluated differences between intercepts by testing for a significant effect of stream on enzyme activity versus respiration regression models.

2.4 Results

2.4.1 Stream Chemistry

In the CPCRW, DOC concentration was variable, tending to be greater in streams draining watersheds with more extensive permafrost, and was greatest during storms. C3, draining a catchment underlain with the greatest permafrost extent, had the highest DOC concentration in 2018 ($p < 0.001$) and 2019 ($p < 0.001$), with an average concentration of 3.5 mg

C L^{-1} for both summers (Table 2.1). C1, draining a catchment underlain with the second highest permafrost extent had the next highest DOC concentrations, with a mean of 2.6 mg C L^{-1} in 2018, and 2.9 mg C L^{-1} in 2019 (Table 2.1). C2 had a mean DOC concentration of 2.2 mg C L^{-1} in 2018 and 2.0 mg C L^{-1} in 2019, and C4 had a mean DOC concentration of 1.9 mg C L^{-1} in 2018 and 1.7 mg C L^{-1} in 2019 (Table 2.1). DOC correlated to stream discharge in all streams (C2: $r^2 = 0.25$, $p < 0.001$, C3: $r^2 = 0.79$, $p < 0.001$, C4: $r^2 = 0.52$, $p < 0.001$).

DOC composition was variable among streams, and inorganic P and ammonium concentrations were low across streams. C1 had the highest average $SUVA_{254}$ at 3.8 L mg C^{-1} m^{-1} and 3.7 L mg C^{-1} m^{-1} in 2018 and 2019, respectively ($p < 0.001$), whereas C4 had the lowest mean $SUVA_{254}$, at 2.9 L mg C^{-1} m^{-1} and 3.1 L mg C^{-1} m^{-1} in 2018 and 2019, respectively ($p < 0.001$; Table 2.1). Nitrate concentration in streams of the CPCRW ranged from 0.2 - 0.9 mg N L^{-1} and varied two-fold among streams. C1 had a significantly lower mean nitrate concentration than the other streams ($p < 0.001$; Table 2.1). Ammonium concentration was at least two-orders of magnitude lower than nitrate concentration, at about 25 μg N L^{-1} across streams. SRP concentration was also low in all streams, ranging from 0.6 - 9 μg P L^{-1}, and did not vary between streams.

2.4.2 Water column and sediment enzyme activity

Median sediment and water column PHOS, BG, NAG, and LAP activities did not significantly vary between streams but were temporally variable. Water column enzyme activities varied by at least 10-fold, whereas sediment enzyme activities varied two to six-fold throughout the summer (Table 2.2). Sediment and water column enzyme activities were uncorrelated to each other and enzyme activity ratios differed between sediment and water

column communities (Fig. 2.2), indicating a difference in microbial organic matter use. However, both water column and sediment enzymes were dominated by PHOS activity (Fig. 2.2), suggesting a common pattern of high organic P processing relative to C and N processing enzymes (Fig. 2.2). The only significant between-stream difference in mean ectoenzyme activity ratios was sediment BG:PHOS, which was higher in C2 than C1 ($p < 0.01$) or C3 ($p < 0.01$). POX activity, only measured in the water column, was at least 1000-fold higher than all other measured enzymes (Table 2.2).

Between streams and over time, variation in enzyme activities was correlated with stream water chemistry. Water column C and N processing enzymes, BG, NAG, and LAP, were all positively correlated with DOC concentration ($p = 0.02$, $p < 0.001$, $p = 0.03$; Table 2.3), whereas PHOS activity was not correlated to DOC and the ratio of PHOS activity relative to C and N processing enzymes decreased with DOC concentration (Table 2.3). Water column PHOS activity was negatively correlated to SRP concentration ($p = 0.03$; Table 2.3). Sediment microbial communities were less responsive to stream chemistry than water column communities, and individual enzyme activities did not significantly correlate to DOC concentration. However, sediment enzyme activity ratios BG:PHOS and (LAP+NAG):PHOS were both positively correlated to SRP concentration ($p < 0.01$, $p = 0.05$; Table 2.4). Stream and sediments enzyme activities did not significantly correlate to $SUVA_{254}$.

2.4.3 NDS Experiment - Microbial Responses to Nutrient and Carbon Amendments

Water chemistry was variable during the 18 days that NDS were deployed, with streams following similar trends as whole summer water chemistry trends. C3 had the highest mean DOC concentration of 5.2 mg C L^{-1} ($p < 0.05$), and highest maximum DOC concentration of 12.2 mg

C L^{-1} (Table 2.1). C1 and C4 also had large increases in DOC concentration during the middle of NDS deployment, with DOC concentration as high as 9.3 mg C L^{-1} in C1, and 4.6 mg C L^{-1} in C4 (Table 2.1). Mean $SUVA_{254}$ was the highest in C1 at 3.8 L mg C^{-1} m^{-1} ($p < 0.001$), compared to an average of 2.4 mg C^{-1} m^{-1} in C3, and 3.0 mg C^{-1} m^{-1} in C4 (Table 2.1). SRP and ammonium concentrations were low in all streams, consistent with season averages (Table 2.1).

Respiration of biofilms from C3 and C4 streams was significantly higher with the labile C treatment than the unamended controls ($p < 0.01$, $p < 0.001$; Table 2.5). Biofilms from the C3 stream had significantly higher respiration with the P + C treatment than C alone ($p < 0.01$; Table 2.5), and biofilms from the C4 stream had significantly higher respiration with the N + C treatment and the P + C treatment compared to C alone ($p < 0.01$, $p < 0.001$; Table 2.5). Respiration of biofilms grown in the C1 stream did not significantly increase with any nutrient or C treatment (Table 2.5). C additions alone significant increased PHOS activity in C3 biofilms ($p < 0.01$) and PHOS and LAP activity in C4 biofilms ($p < 0.001$, $p < 0.01$; Table 2.5). C and P amendments in combination significantly increase NAG and BG activity in C3 biofilms ($p < 0.01$ $p < 0.01$; Table 2.5), while N+P+C amendments significantly increased NAG and BG activity in C4 biofilms ($p < 0.05$, $p < 0.001$). In C1, N+P+C amendments significantly increased BG activity ($p < 0.05$; Table 2.5).

Biofilms grown on NDS disks enhanced with P had distinct ectoenzyme activities relative to biofilms not treated with P. P additions decreased biofilm PHOS activity in C3 ($p < 0.01$) and C4 ($p < 0.01$; Table 2.5), and treatments that included P (P, P+C, N+P+C) generally had higher BG:PHOS and (NAG+LAP):PHOS ratios relative to unamended controls (Table 2.6). PHOS activity was positively correlated to NAG + LAP activity for biofilms grown in all streams (C1: $r^2 = 0.76$, $p < 0.001$, C3: $r^2 = 0.89$, $p < 0.001$, C4: $r^2 = 0.88$, $p < 0.001$; Fig. 2.3), with the slope

of PHOS relative to NAG+LAP significantly lower in P amended than non-P amended biofilms (C1: $p < 0.001$, C3: $p < 0.001$, C4: $p < 0.001$; Fig. 2.3). With greater enzyme activities in the N and C treatments, P amended biofilms had proportionally larger increases in N-acquiring enzymes while non-P amended biofilms had proportionally larger increases in P-acquiring enzymes (Fig. 2.3). Unlike with P additions, N and C additions did not alter allocation towards N, P, and C acquiring enzyme activities; treatments that did not include P had no significant effects on ectoenzyme activity ratios relative to unamended controls (Table 2.6).

LAP, BG, and NAG activities were all positively related to respiration (LAP: $r^2 = 0.77$, $p < 0.001$, BG: $r^2 = 0.52$, $p < 0.001$, NAG: $r^2 = 0.40$, $p < 0.001$; Fig. 2.4). Enzyme activity regressed against respiration revealed that C1, C3, and C4 streams differed in organic nutrient and C acquisition efforts relative to respiration. C1 biofilms had a significantly lower y-intercept for LAP activity against respiration than C3 ($p < 0.001$) and C4 ($p < 0.001$; Fig. 2.4). C3, the stream with the highest mean DOC concentration (Table 2.1), had biofilms with a higher y-intercept for BG activity against respiration than C1 ($p < 0.001$) and C4 ($p < 0.001$; Fig. 2.4). Because P-treated biofilms had lower PHOS activity than non-P treated biofilms, PHOS and respiration were positively correlated when P treatments were excluded ($r^2 = 0.75$, $p < 0.01$; Fig. 2.5). Streams varied in slope for PHOS versus respiration ($p < 0.01$; Fig. 2.5), and biofilms from C3 had the highest slope.

2.5 Discussion

2.5.1 P Limitations

Enzyme activities in streams of the CPCRW indicated P and labile C limitations, similar to patterns observed in other high latitude streams (Mann et al. 2012, Pastor et al. 2019), and

other studies done at the CPCRW (Mutschlecner et al. 2018). Water column and sediment PHOS, LAP, BG, and NAG activities in the streams of the CPCRW were within range of other reported freshwater water column and sediment activities (Sinsabaugh et al. 2009, Hill et al. 2010b, Sinsabaugh and Shah 2012). However, enzyme ratios differed from global mean ratios with disproportionately high investment in PHOS activity (Sinsabaugh et al. 2008, 2009). Globally, BG:PHOS:(NAG+LAP) enzyme activity ratios average at approximately 1:1:1, empirically linked to an equilibrium relating environmental C:N:P availability, microbial biomass composition, and carbon and nutrient use efficiencies (Sinsabaugh et al. 2008, 2009). Deviations from this equilibrium have since been used to resolve resource limitations of freshwater microbial communities (Hill et al. 2010a, Burpee et al. 2016, Pastor et al. 2019). Water column enzyme activity ratios were similar to other P-limited streams (Mann et al. 2014), with high investment towards PHOS activity at the CPCRW likely due to low P availability (Sinsabaugh et al. 2008).

High PHOS activity at the CPCRW suggests that microbes are investing in organic P processing to meet P demand. Our NDS experiment demonstrated that PHOS activity decreased in response to inorganic P additions across streams (Table 2.5), and an inverse correlation between PHOS activity and ambient SRP concentration (Table 2.3, Table 2.4) suggests that inorganic P controlled organic P utilization at an ecologically relevant scale. Organic P is found in aquatic environments as inositol phosphates, sugar phosphates, phospholipids, nucleic acids, and phosphoproteins (Paytan and Mclaughlin 2007), with phospholipids and nucleic acids, processed by PHOS, composing the labile organic P pool (Sinsabaugh and Shah 2012). Organic P can make up a large portion of total bioavailable P and be important in overcoming microbial P limitations (Nausch and Nausch 2007, Mutschlecner et al. 2017, Soares et al. 2017). In streams

of the CPCRW, dissolved organic P (DOP) comprises about 40% of total dissolved P (Table 2.1). Microbial use of this DOP is dependent on composition and bioavailability, as well as PHOS production and the effectiveness of PHOS processing (Olsson et al. 2012, Mann et al. 2014, Thompson and Cotner 2018). Changes in organic P demand with inorganic P availability will affect energy and nutrients invested towards PHOS activity and consequently resources available towards other enzyme production and growth (Sinsabaugh and Moorhead 1994, Moorhead et al. 2013).

Relatively high investment in PHOS activity suggests P-limitation in streams of the CPCRW (Fig. 2.2), however, inorganic P addition alone did not limit respiration of biofilms grown in any stream we measured (Table 2.5). The lack of a P-induced response in respiration may be due to an increase in carbon use efficiency (Smith and Prairie 2004). Under nutrient limiting conditions microbes process C that cannot be used for growth, resulting in waste respiration (Schimel and Weintraub 2003). CPCRW streams have low SRP concentration (Table 2.1), suggesting a stoichiometric imbalance and thus high rate of respiration relative to growth. Additionally, the decrease in PHOS activity with P additions that we observed may have led to a decrease in C processing. Microbes must respire C to obtain energy required to produce PHOS (Schimel and Weintraub 2003) and, although PHOS activity targets the acquisition of P, PHOS processing can make C compounds available potentially fueling respiration (Hoppe and Ullrich 1999, Steenbergh et al. 2011, Spohn and Kuzyakov 2013).

2.5.2 Labile C as control on DOM processing

Labile C availability controlled rates of DOM processing. C and nutrient manipulations demonstrated that labile C limited or co-limited respiration and the C and N processing enzyme

activities of biofilms grown in streams of the CPCRW (Table 2.5). C and N additions did not affect ectoenzyme activity stoichiometry (Table 2.6), suggesting that microbes were not changing resource allocation patterns but instead increasing rates of DOM processing (Sinsabaugh and Moorhead 1994). Inorganic nutrient additions only increased C processing when biofilms were also supplemented with a labile C source (Table 2.5, Fig. 2.4), suggesting that C limitations needed to be relieved to increase nutrient demand and thus for nutrient additions to stimulate C processing (Bernhardt and Likens 2002). We amended NDS with acetate, a highly labile from of DOC, whereas ambient stream DOC is heterogeneous, complex, and substantially less bioavailable (Mutschlecner et al. 2018).

C limitation likely resulted from DOC compositional constraints on microbial processing. POX activity in water column microbial communities was up to1000 times higher than BG activity, the other C processing enzyme measured (Table 2.2). Enzyme studies in other high latitude streams have reported similarly high POX activity relative to BG activity (Mann et al. 2014). POX catalyzes lignin degradation, acquiring a less energetically favorable C source than cellulose, and the ratio of BG:POX is negatively correlated to the proportion of recalcitrant DOC (Sinsabaugh and Shah 2011). Streams of the CPCRW have low DOC concentration with the majority of C recalcitrant to microbial processing (Balcarczyk et al. 2009, Mutschlecner et al. 2018). Low BG activity is consistent with evidence that there is little bioavailable DOC substrate such as cellulose available, and high POX activity relative to BG activity further suggests a highly recalcitrant C source available for microbial processing.

Allochthonous C supply is important in supporting stream heterotrophic communities (Tank et al. 2010, Roiha et al. 2016), especially in forested headwater streams with strong links to terrestrial flowpaths and low primary production (Fisher and Likens 1973). We found that

water column C and N processing enzyme activities increased with ambient stream DOC concentration (Table 3), suggesting enzymatic processing of DOM as it becomes available. Increases in DOM may have led to a proportional increases in enzyme-degradable substrate availability, prompting microbes to upregulate C and N processing enzymes (Williams et al. 2012). Additionally, an increase in available C and nutrients supplied by DOM may have fueled enzyme production and subsequently C processing (Allison and Vitousek 2005). In streams of the CPCRW, DOM concentration increases during storms due to the flushing of terrestrial flow paths (Petrone et al. 2006), and in other catchments can lead to a compositional shift in DOM (Buffam et al. 2001, Wiegner et al. 2009, Singh et al. 2015). Our data indicated that enzyme activities and $SUVA_{254}$ were not correlated, suggesting that enzyme activities were not sensitive to DOM composition. However, it's possible that variation in DOM composition not detected from measuring $SUVA_{254}$ influenced enzyme activities.

2.5.3 Microbial Utilization of Organic Matter

Proportionally high LAP activity indicated high amino acid processing across streams, with biofilms grown in different streams varying in relative investment towards peptide, chitin, and cellulose processing. Biofilms grown on NDS disks and water column microbial communities both had higher LAP activity than other measured C and N processing enzymes (Table 2.2, Fig. 2.4). Amino acids can support a large portion of microbial metabolism as a source of C and N (Jorgensen et al. 1993, Keil and Kirchman 1999), and amino acid components in DOM can be a strong predictor of DOM bioavailability (Amon et al. 2001, Balcarczyk et al. 2009). Relatively high peptidase processing in the CPCRW suggests that amino acids are an important source of resources for water column microbial communities (Harbott and Grace 2005). Additionally, NDS biofilms grown in different streams had different C and N processing

enzyme activities relative to respiration, suggesting a difference in organic substrates to support total C processing. Higher BG activity relative to respiration in C3 compared to C1 or C4 may reflect that C3 stream biofilms processed more cellulose to meet metabolic C demand (Fig. 4). Similarity, C1 stream biofilms had the lowest LAP activity relative to respiration, suggesting lower peptidase processing in C1 biofilms compared to other streams in order to meet C and N requirements (Fig. 4).

Consistent with global trends, we observed that water column enzymes exhibited higher relative investment towards PHOS and LAP, and lower relative investment towards BG and NAG compared to enzyme activities of sediment biofilms (Fig. 2.2, Table 2.2). Water column and sediment microbial communities vary in resource availability and biomass stoichiometry, and consequently ectoenzyme activity ratios (Sinsabaugh et al. 2010, Sinsabaugh and Shah 2012, Hill et al. 2012). P has a high sorption capacity and can thus accumulate in sediment. In general, sediment microbial communities invest less into PHOS activity compared to water column communities, presumably due to higher P availability (Sinsabaugh and Shah 2012, Hill et al. 2012). Water column and sediment communities also generally rely on different organic matter compounds for C acquisition. Global average ratios indicate that water column microbial communities express higher LAP activity to meet C requirements, while sediment communities rely more on cellulose processing (Sinsabaugh and Shah 2012). Organic matter accumulates in sediment, leading to proportionally higher substrate that induces BG and NAG processing (Romani and Sabater 2001).

2.5.4 Future Changes:

Our results suggest that microbial processing of DOM in boreal forest streams with low P concentration is sensitive to variation in inorganic P availability. Stream P concentration has decreased in Arctic Alaskan streams in the past thirty years, perhaps due to warming leading to increased terrestrial production and P demand and decreased P export to streams (Kendrick et al. 2018). However contrasting evidence also suggests that P concentration in high latitude streams may increase with warming and permafrost thaw, due to mobilization of P from the mineral soil layer and increased groundwater contributions to streams (Hobbie et al. 1999, Frey et al. 2007a, Frey and McClelland 2009). Small changes in P availability can have dramatic ecosystem effects in low nutrient streams. In Arctic Alaska, stream-reach P additions led to increased primary production, and a shift to greater use of allochthonous C by heterotrophs (Peterson et al. 1985). P-limited boreal forest streams will likely also experience alterations in stream function as P availability changes.

DOM quality is an important control on DOM cycling. In boreal forest streams, as much as 95% of DOM is unavailable to microbial processing due to compositional constraints (Balcarczyk et al. 2009). DOM composition and C bioavailability can also control the extent that inorganic nutrients stimulate microbial processing of DOM (Ardon and Pringle 2007). Consistent with our study, recent work in streams of the CPCRW has also demonstrated C co-limiting heterotrophic activity, with ambient stream DOC bioavailability, measured as the proportion of DOC consumed by microbes in lab incubations, important in determining heterotrophic responses to inorganic nutrient additions (Weaver 2019). As permafrost thaw alters vegetation and soil properties, DOM compositional changes and acclimation of microbial communities to process DOM of varied composition will likely control C and nutrient cycling (Frey et al. 2016, D'Andrilli et al. 2019).

Our results suggest that inorganic nutrients and labile C co-limited microbial processing of DOM. There is evidence that permafrost thaw will lead to increased DOC concentration and increased nitrate concentration in streams (Striegl et al. 2005, McClelland et al. 2007). Inorganic P concentration will likely also be affected by permafrost degradation but the direction of change is uncertain (Frey et al. 2007b, Frey and McClelland 2009, Kendrick et al. 2018). Projected increases in DOC and inorganic nutrients suggest that microbial processing of DOM will increase. Altered DOC processing has the potential to influence resource export and consequently ecological function of downstream systems (McClelland et al. 2014). Additionally, altered resource availability at the base of the aquatic food web will likely affect higher trophic levels supported by microorganisms (Kendrick et al. 2018).

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2.7 Figures

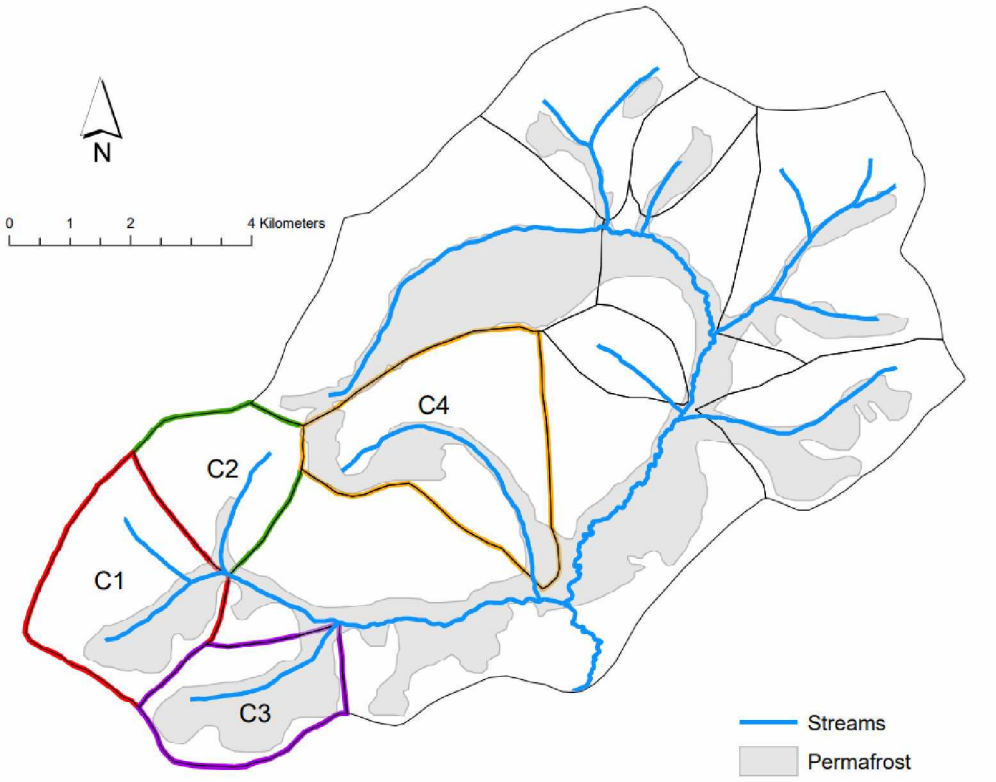

Figure 2.1: Map of Caribou Poker Creeks Research Watershed. Catchment boundaries are highlighted, and grey shading shows permafrost extent within each headwater stream catchment.

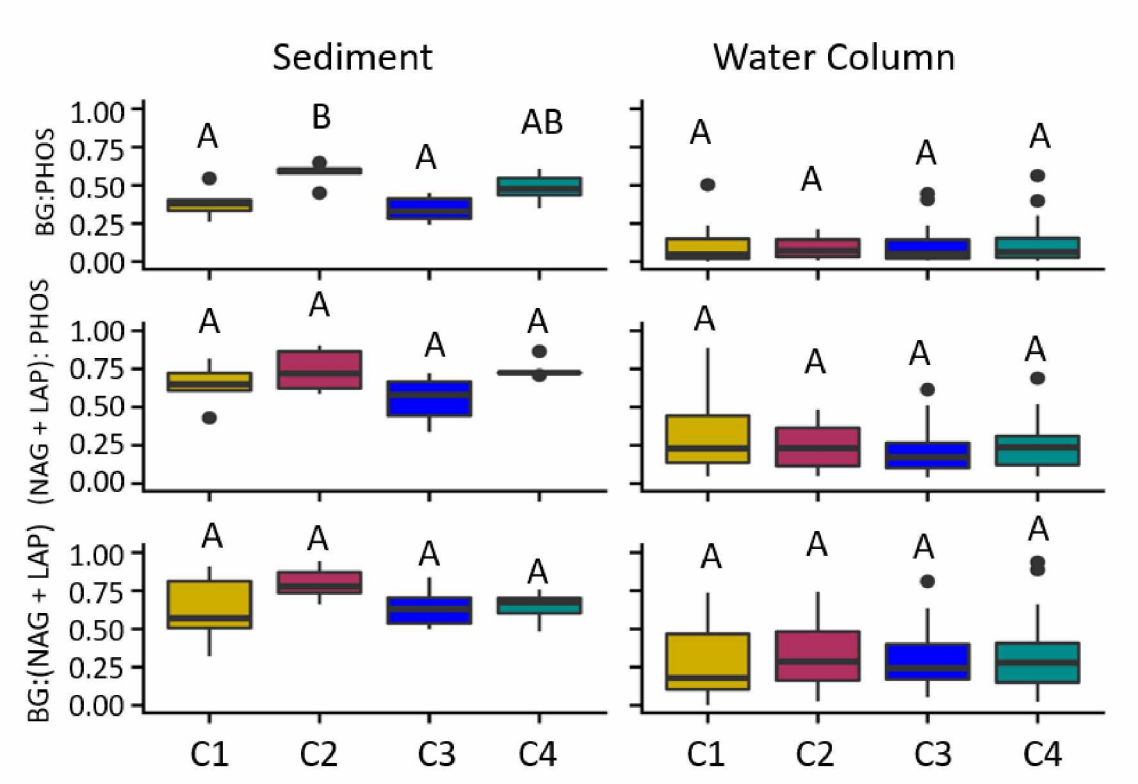

Figure 2.2: Ratios of nutrient and carbon acquiring enzymes. Boxplot of enzyme activity ratios from sediment samples collected in summer 2019 (n=6), and water column samples collected form 2018 and 2019 (n=17). Panels show C:P acquiring enzymes, C:N acquiring enzymes, and N:P acquiring enzymes. BG=Beta glucosidase, PHOS=Phosphatase, NAG= β-1,4- N-acetylglucosaminidase, LAP= Leucine Amino Peptidase. The center lines, box extent, error bars and points indicate the 50th percentile (median), the 25th and 75th percentile (interquartile range), the 95% confidence intervals, and outliers, respectively. Different letters above plots represent significant differences between streams ($p < 0.05$).

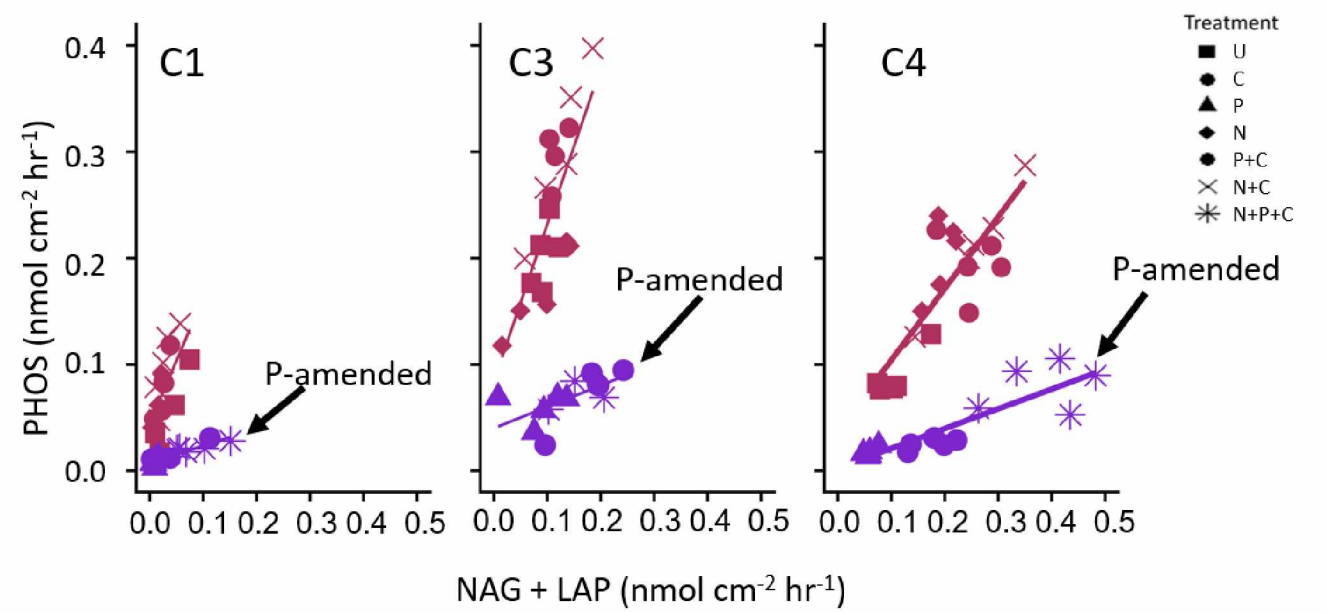

Figure 2.3: N acquring versus P acquiring enzmye activity ratio plots of NDS biofilms. Treatments are grouped into P-ammeded (P, P+C, N+P+C) and non-P ammended (U, C, N, N+C), with different linear regression lines fitted to each group. Each point represents a single measurement, with five measurements per treatment. C1 P amended: y=0.16x+0.01, C1 non-P amended: y=1.22x+0.04, C3 P amended: y=0.20x+0.03, C3 non-P amended: y=1.42x+0.08, C4 P amended: y=0.19x+0.00, C4 non-P amended: y=0.67x+0.03.See figure 2 for full enzyme names.

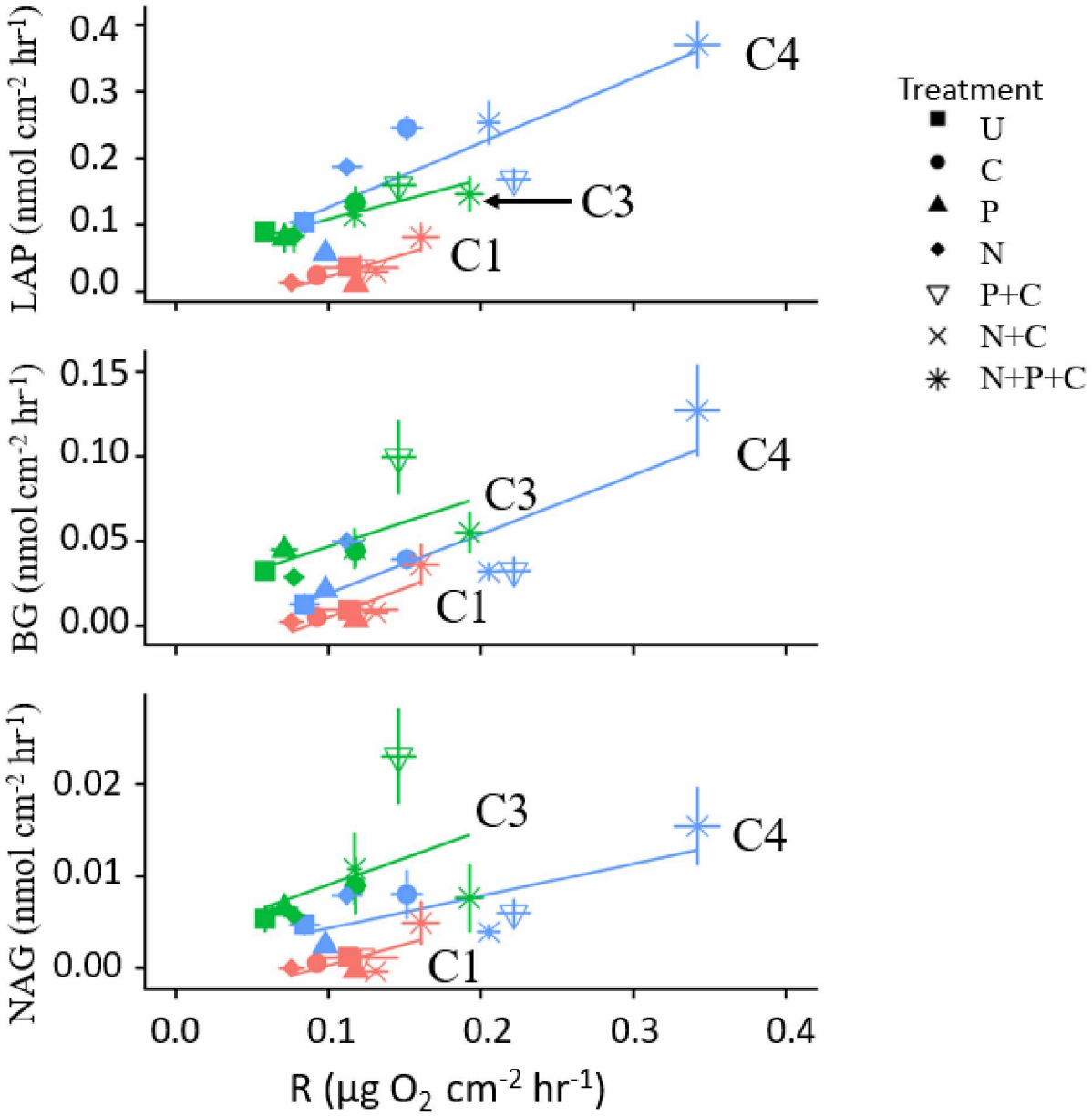

Figure 2.4: C and N processing enzyme activity plotted against respiration (R). Each point represents mean enzyme activity (+/- se) against mean respiration (+/- se) for each nutrient treatment, with five replicates per treatment. Intercepts significantly differ, reflecting between-stream differences in enzyme activity relative to respiration. See figure 2 for full enzyme names.

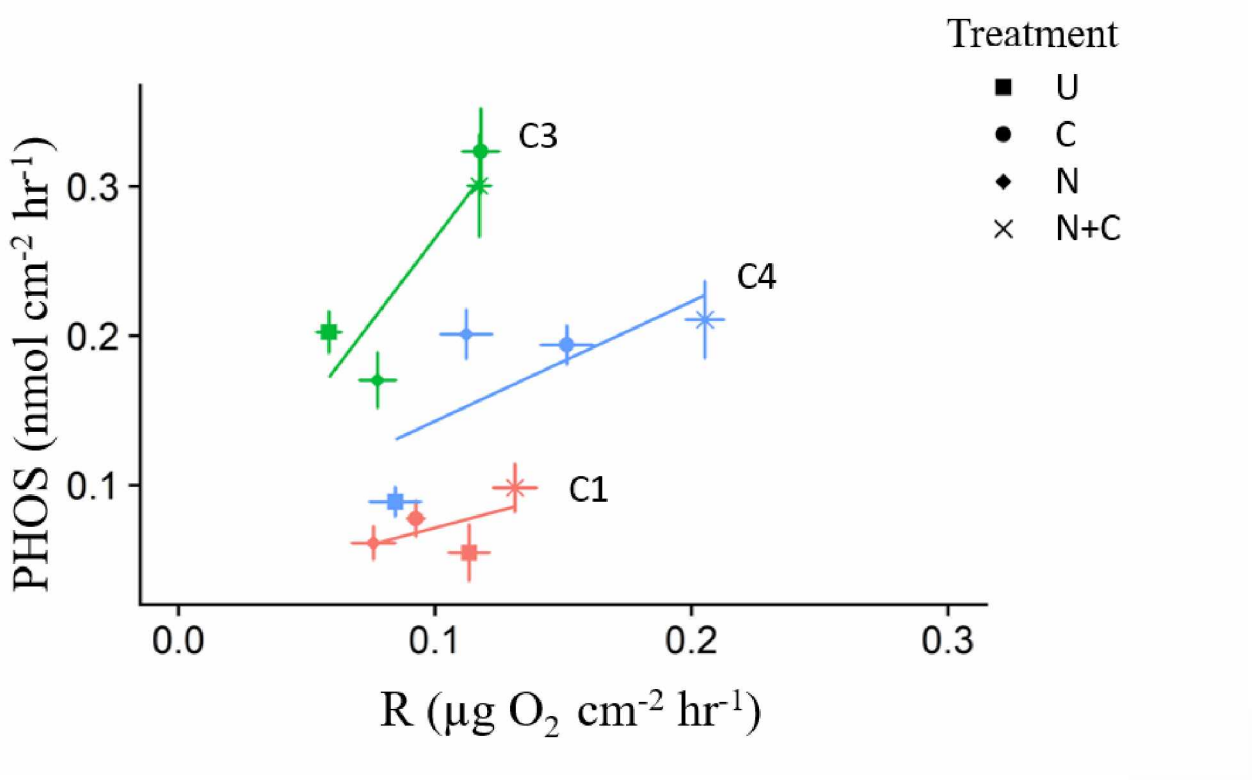

Figure 2.5: PHOS activity plotted against respiration (R), excluding P treaments. Demonstrates positive relationshion between PHOS and respiration with carbon and N ammendments. Each point represents mean PHOS activity (+/- se) and mean respiration (+/- se), with five reps per treatment.

2.8 Tables

Table 2.1: Water chemistry and discharge for Caribou Poker Creaks Research Watershed. Mean and range of DOC and nutrient concentrations, DOC optical properties, and discharge for 2018, 2019, and during NDS deployment.

		DOC mg C L^{-1}	DON µg N L^{-1}	DOP µg P L^{-1}	SUVA L mg C^{-1} m^{-1}	SR	NO_3 mg N L^{-1}	NH_4 µg N L^{-1}	SRP µg P L^{-1}	Discharge L s^{-1}
2018	C1	2.6 (1.9-4.3)	110.1 (60.2-113.4)	1.5 (0.5-5.0)	3.7 (3.1-4.3)	---	0.3 (0.2-0.4)	25.8 (0.0- 191.6)	2.5 (0.6-5.0)	* ---
	C2	2.7 (1.5-3.5)	90.5 (30.2-140.8)	2.0 (0.2-7.6)	3.3 (2.1-4.0)	---	0.6 (0.5-0.7)	24.7 (0.0- 134.2)	3.6 (0.9-14.6)	63.0 (28.7-102.0)
	C3	3.5 (2.4-5.2)	120.6 (60.2-160.6)	2.9 (0.2-5.5)	3.3 (2.7-3.9)	---	0.6 (0.5-0.6)	30.5 (0.00-184.1)	3.3 (0.6-9.9)	75.1 (23.9-210.3)
	C4	1.9 (1.3-2.9)	90.4 (30.9-130.0)	1.8 (0.1-5.6)	2.7 (2.3-3.3)	---	0.7 (0.6-0.7)	25.9 (0.0-212.4)	3.0 (1.2-7.1)	115.3 (60.3-205.5)
2019	C1	2.8 (1.3-9.3)	150.8 (40.2-440.6)	2.7 (1.5-6.6)	3.7 (3.1-4.1)	0.5 (0.4-0.6)	0.3 (0.2-0.4)	31.2 (0.0- 119.1)	2.8 (1.6-4.7)	*134.5 (45.3-306.3)
	C2	2.0 (1.1-4.1)	100.6 (30.4-240.4)	2.3 (0.7-6.4)	3.7 (3.1-4.2)	0.4 (0.2-0.6)	0.5 (0.4-0.7)	31.1 (0.0- 86.7)	2.8 (1.9-4.7)	44.1 (8.5-180.2)
	C3	3.5 (1.4-12.2)	120.9 (30.1-350.3)	3.3 (1.4-6.4)	3.2 (2.7-4.0)	0.5 (0.3-0.6)	0.7 (0.4-0.9)	25.5 (0.0- 117.1)	2.8 (0.9-4.3)	89.1 (13.3-578.3)
	C4	1.7 (0.6-4.6)	90.6 (20.2-260.8)	1.5 (0.3-3.9)	3.1 (2.1-4.1)	0.3 (0.1-0.6)	0.6 (0.5-0.8)	26.4 (0.0- 141.1)	3.0 (1.9-4.7)	93.3 (42.2-515.3)
NDS	C1	3.6 (1.3-9.3)	140.0 (40.1-440.0)	2.1 (0.7-4.2)	3.4 (3.1-4.0)	0.6	0.3 (0.2-0.4)	34.0 (0.0- 73.1)	3.0 (2.5 3.4)	*173.7 (78.4-269.0)
	C3	5.2 (1.7-12.2)	100.8 (30.9-240.6)	2.7 (0.5-4.1)	3.2 (2.5-3.6)	0.6	0.7 (0.5-0.9)	27.8 (0.0- 53.1)	3.2 (2.2 4.3)	144.4 21.2-578.3
	C4	1.8 (0.8-4.6)	40.5 (20.7-200.1)	2.2 (1.4-3.5)	2.8 (2.1-3.2)	0.6	0.7 (0.5-0.8)	15.5 (0.0- 55.8)	3.5 (1.9 4.7)	92.6 (30.5-515.3)

*C1 is the only stream where discharge was not measured continuously throughout the summer field season; stream discharge was manually measured biweekly in 2019.

Table 2.2: Mean and range enzyme activities of water column samples (2018 and 2019) and sediment samples (2019). Sediment and water column enzyme activities did not significantly vary between streams. BG=Beta glucosidase, PHOS=Phosphatase, NAG= β-1,4- N-acetylglucosaminidase, LAP= Leucine Amino Peptidase

	Stream	PHOS nmol hr^{-1} ml^{-1}	LAP nmol hr^{-1} ml^{-1}	BG nmol hr^{-1} ml^{-1}	NAG nmol hr^{-1} ml^{-1}	POX nmol hr^{-1} ml^{-1}
Water Column 2018	C1	0.11 (0.02-0.25)	0.024 (.002-.044)	0.005 (.000-.020)	0.004 (.001-.007)	3.81 (2.49-5.21)
	C2	0.13 (0.04-0.28)	0.024 (.007-.032)	0.007 (.001-.014)	0.003 (.001-.005)	5.64 (3.61-7.23)
	C3	0.14 (0.04-0.32)	0.021 (.006-.032)	0.007 (.003-.021)	0.003 (.000-.005)	4.85 (3.27-7.27)
	C4	0.16 (0.07-0.28)	0.023 (.004-.041)	0.009 (.000-.046)	0.002 (.001-.004)	9.89 (7.78-12.4)
Water Column 2019	C1	0.18 (.044-0.54)	0.051 (.019-.098)	0.033 (.002-.121)	0.008 (.002-.0331)	5.85 (4.41-8.77)
	C2	0.20 (.066-.642)	0.044 (.015-.102)	0.33 (.003-.139)	0.005 (.001-.010)	8.51 (7.04-11.3)
	C3	0.20 (.061-.633)	0.035 (.018-.067)	0.022 (.001-.081)	0.006 (.001-.013)	7.78 (6.14-12.2)
	C4	0.16 (.084-.529)	0.035 (.015-.066)	0.023 (.004-.051)	0.003 (.000-.019)	12.8 (10.5-16.0)
		PHOS nmol hr^{-1} g^{-1}	**LAP** nmol hr^{-1} g^{-1}	**BG** nmol hr^{-1} g^{-1}	**NAG** nmol hr^{-1} g^{-1}	**----**
Sediment 2019	C1	26.3 (17.0-44.3)	10.55 (8.67-11.6)	10.63 (4.41-20.5)	5.31 (2.23-12.3)	
	C2	24.7 (16.1-28.1)	12.39 (7.18-16.3)	14.39 (7.26-22.8)	5.37 (2.30-12.6)	
	C3	23.3 (17.7-28.4)	10.02 (5.47-14.6)	8.20 (4.96-13.6)	2.89 (1.40-4.57)	
	C4	17.9 (8.3-28.4)	9.49 (5.10-13.8)	9.02 (2.90-15.6)	3.86 (0.92-6.81)	

Table 2.3: Spearman rank correlation of water column ectoenzyme activities and ectoenzyme ratios versus water chemistry from CPCRW headwater streams. Enzyme and water chemistry samples were collected bi-weekly throughout the 2018 and 2019 summers. P values with asterisks represent significant levels (*<.05, **<.01, ***<.001). See Table 2.2 for full enzyme names.

Enzyme	Chemistry Parameter	DF	P value	r_s
PHOS	SRP	52	0.03*	-0.32
BG	DOC	62	0.02*	+0.29
LAP	DOC	62	0.03*	+0.27
NAG	DOC	61	<0.001***	+0.43
BG:PHOS	DOC	57	<0.01**	+0.36
LAP+(NAG:PHOS)	DOC	58	0.01*	+0.31
BG:(LAP+NAG)	DOC	61	0.04*	+0.25

Table 2.4: Spearman rank correlation of sediment ectoenzyme activity ratios versus water chemistry from CPCRW headwater streams. Enzyme and water chemistry samples were collected bi-weekly throughout the 2019 summer. P values with asterisks represent significant levels (*<.05, **<.01, ***<.001). See Table 2.2 for full enzyme names.

Enzyme Ratio	Chemistry Parameter	DF	P value	r_s
BG : PHOS	SRP	14	>0.01**	+0.61
(LAP + NAG):PHOS	SRP	14	0.05	+0.46

Table 2.5: NDS biofilm enzyme activities and respiration (R) responses to nutrient and C amendments. Each treatment included 5 replicates. Arrows indicate direction of significant response relative to unamended control (ANOVA, $p < 0.05$) and Ns indicates no significant response relative to unamended control. An X after treatments with multiple nutrient and C additions indicates that additional nutrient or C caused response significantly higher than response with single nutrient or C amendment. See Table 2.2 for full enzyme names.

Site	Enzyme or R	Unamended (nm hr^{-1} cm^{-2})	Treatment					
			+C	+N	+P	+N+C	+P+C	+N+P+C
C1	PHOS	0.054	Ns	Ns	Ns	Ns	Ns	Ns
	BG	0.0094	Ns	Ns	Ns	Ns	Ns	↑
	NAG	0.0012	Ns	Ns	Ns	Ns	Ns	Ns
	LAP	0.037	Ns	Ns	Ns	Ns	Ns	Ns
	R		Ns	Ns	Ns	Ns	Ns	Ns
C3	PHOS	0.202	↑	Ns	↓	↑	↓	↓
	BG	0.032	Ns	Ns	Ns	Ns	↑	Ns
	NAG	0.0054	Ns	Ns	Ns	Ns	↑	Ns
	LAP	0.090	Ns	Ns	Ns	Ns	Ns	Ns
	R		↑	Ns	Ns	↑	↑ X	↑ XX
C4	PHOS	0.089	↑	↑	↓	↑	↓	Ns
	BG	0.013	Ns	Ns	Ns	Ns	Ns	↑
	NAG	0.005	Ns	Ns	Ns	Ns	Ns	↑
	LAP	0.104	↑	Ns	Ns	↑	Ns	↑ X
	R		↑	Ns	Ns	↑ X	↑ X	↑ XX

Table 2.6: NDS biofilm enzyme activity ratio responses to nutrient and C treatments. Each treatment included 5 replicates. Arrows denote direction of significant effect on enzmye activity ratios relative to unamended control ($p < 0.05$), and Ns represents no significant response realtive to unamended control. An X after treatments with more than one nutrient or C addition indicates that additional nutrient caused a response significantly higher than significant response with single nutrient or C addition. See figure 2 for full enzyme names.

Site	Enzyme Ratio	Treatment					
		+C	+N	+P	+P+C	+N+C	+N+P+C
C1	BG:PHOS	Ns	Ns	Ns	↑	Ns	↑
	BG:(NAG + LAP)	Ns	Ns	Ns	Ns	Ns	Ns
	(NAG + LAP):PHOS	Ns	Ns	Ns	Ns	Ns	↑
C3	BG:PHOS	Ns	Ns	↑	↑ X	Ns	↑
	BG:(NAG + LAP)	Ns	Ns	Ns	Ns	Ns	Ns
	(NAG + LAP):PHOS	Ns	Ns	↑	↑ X	Ns	↑
C4	BG:PHOS	Ns	Ns	↑	↑	Ns	↑
	BG:(NAG + LAP)	Ns	Ns	↑	Ns	Ns	↑
	(NAG + LAP):PHOS	Ns	Ns	↑	↑ X	Ns	↑

Chapter 3: General Conclusion

3.1 Conclusions

Our results suggest that inorganic phosphorous (P) and labile carbon (C) affected microbial processing of DOM throughout a boreal forest watershed underlain with discontinuous permafrost. Inorganic P availably inversely controlled microbial P demand and investment towards organic P acquisition. Labile C limited or co-limited rates of C mineralization and the activity of C processing ectoenzymes. This work contributes to a broader research pool evaluating high latitude stream resource limitations and how changes in resource availability will impact the processing of C and nutrients through stream networks (McClelland et al. 2007, Mann et al. 2012, 2014, Kendrick et al. 2018, Pastor et al. 2019).

In addition to stoichiometric constraints, temperature can also constrain microbial processing of DOM. High latitude streams underlain with permafrost are cold year-round, limiting metabolism. Climate warming and permafrost degradation are leading to increased stream temperature (Rice and Jastram 2015, Docherty et al. 2019). Increased inorganic nutrient availability will likely increase the extent that warmer temperature stimulates biotic processing of DOM (Berggren et al. 2010). Additionally, warmer temperatures selects for microbial communities with higher nutrient use efficiencies, proportion of nutrients processed by microbes that are acquired into biomass (Hall et al. 2009). This leads to temperature nutrient interactions, making temperature an important variable when considering permafrost thaw controls on C and nutrient cycling.

Seasonal variation in water chemistry and stream temperature causes temporal variation in resource limitation. Metabolic constraints during cold winter months can limit heterotrophic metabolism (Burrows et al. 2017). During spring, a large flush of DOM enters streams with

snowmelt. This DOM is generally labile relative to other times of the year (Holmes et al. 2008, Wickland et al. 2012, Mutschlecner et al. 2018), and can be used to overcome C and nutrient constraints on decomposition. A large portion of annual water, nutrient, and DOC flux occurs during spring snowmelt (Kane et al. 2004, McNamara et al. 2008), making it a crucial time for studies of microbial processing of DOM.

Changes in water chemistry can alter microbial community composition (Lee et al. 2017). Microbial resource demand, evaluated by ectoenzyme activity stoichiometry, is constrained globally over a wide range in resource availability, suggesting adaptations of microbial community composition in order to minimize resource demand (Sinsabaugh et al. 2008). With changes in nutrients and C, microbes with stoichiometric requirements better suited to stream resource availability and specialized to process available DOM will dominate streams (D'Andrilli et al. 2019). Evaluating changes in stream water chemistry in relation to microbial community composition and resource demand is necessary in resolving how water chemistry controls microbial processing of DOM (Hall et al. 2011).

Predicting the fate of terrestrial organic matter upon entering streams is important in understanding broader ecosystem processes. Microbial biomass production often makes up the base of the food web, fueling higher trophic levels (Kendrick et al. 2018). Additionally, C transformations and microbial respiration of C will create greenhouse gas emissions. Release of permafrost C and subsequent microbial processing can act as a positive climate warming feedback (Schuur et al. 2015). Unprocessed DOM will be transported downstream, potentially fueling production of downstream systems (Dunton et al. 2006, McClelland et al. 2014).

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